Comprehensive re-assessment of causality of *ABCC6* missense variants associated with pseudoxanthoma elasticum

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Introduction

Pseudoxanthoma elasticum (PXE) is an ectopic mineralization disorder affecting the elastic fibers of the skin, the eye and the cardiovascular system. It is caused by biallelic mutations leading to loss-of-function of the *ABCC6* gene. To date, over 400 genetic variants have been reported in *ABCC6*. However, high phenotypic variability, a lack of genotype-phenotype correlations and contemporary population genomics raise the question whether all these variants are pathogenic. Especially for missense substitutions it is challenging to predict the impact on protein function. We therefore systematically evaluated previously reported and novel ***ABCC6* missense variants** and classified them according to pathogenicity, using a standardised approach.

Sherloc classifies variants based on point thresholds

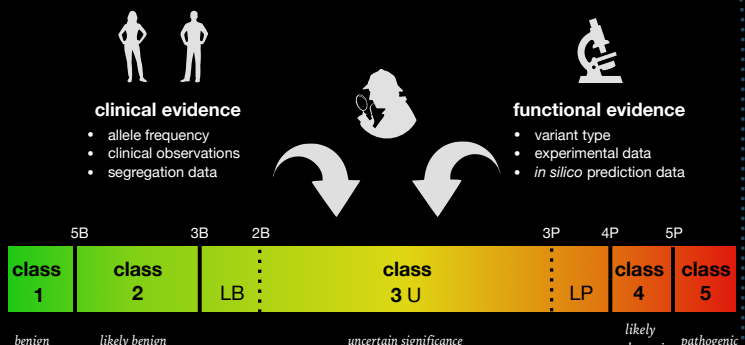

Fig 1. Evaluation and scoring of clinical and functional evidence with benign (B) or pathogenic (P) points leads to classification of the variant based on its total score. Subclasses of class 3, that originally do not exist in Sherloc, were created to highlight those variants on the verge of class 2 (class 3 LB) or 4 (class 3 LP). If conflicting evidence was used, a hierarchical approach (not shown) was followed to determine which evidence type is more decisive.

Most ClinVar variants are reported as pathogenic

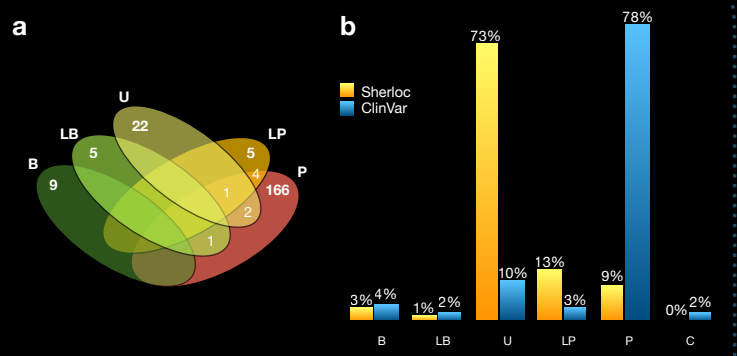

Fig 3. (a) The Venn diagram represents the number of variants reported as benign (B), likely benign (LB), uncertain (U), likely pathogenic (LP) and/or pathogenic (P) in ClinVar (215 in total). Some variants have multiple interpretations and are considered conflicting when (L)B interpretations co-occur with (L)P interpretations or when U co-occurs with (L)B/(L)P. **(b)** In contrast to the reported interpretations, the majority of these variants is classified as U using Sherloc. The percentage of conflicting (C) results in Sherloc is 0, because conflicting evidence is handled by a hierarchical approach.

Conclusions

Evaluation of *ABCC6* missense variants using the most recent and comprehensive criteria reveals an **overestimation of (likely) pathogenic variants in literature and databases**. Data that are missing for many variants are experimental validation and - surprisingly - segregation data. Our results underline that variant classification should be done systematically and with caution, as variant interpretation has **important consequences for patients and carriers identified via familial or expanded carrier screening.** The high number of class 3 variants confirms the need for functional testing to prove or refute their causality before returning them to patients.

Methodology

We evaluated all *ABCC6* missense variants from ClinVar, from literature and novel variants from in-house patient screenings. In total, 235 variants were analysed using the numerical score-based variant classification system **Sherloc** (Nykamp *et al.* 2017). Clinical and functional evidence were scored with benign (B) or pathogenic (P) points using hierarchical decision trees and classification was based on the variant's total score (Fig 1). To distinguish between different types of class 3 variants we created subclasses for variants with a likely benign (LB), likely pathogenic (LP) or unclear (U) tendency (Fig 2b). We evaluated the same variants using guidelines from Sherloc's forerunner, the ACMG-AMP classification system (Richards *et al.* 2015), and compared the outcome of both (Fig 2a). The interpretation of variants submitted in ClinVar was also compared with the corresponding result after applying Sherloc (Fig 3). Finally, the distribution of variants along the protein domains was investigated (Fig 4).

Majority of *ABCC6* missense variants are class 3

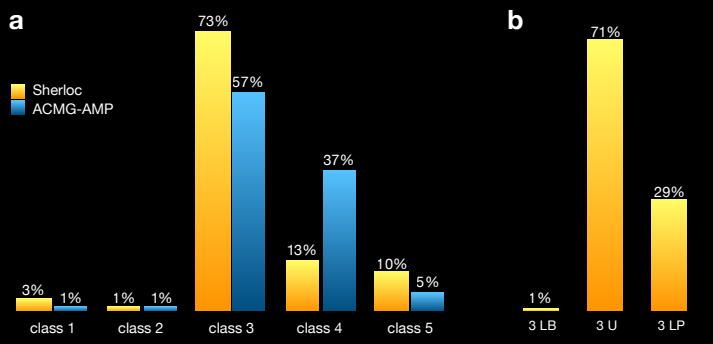

Fig 2. (a) 235 variants were classified using the Sherloc (yellow) or ACMG-AMP (blue) framework. Class 3 variants were was most abundant, followed by class 4 & 5. Few class 1 or 2 variants were found. **(b)** Subclassification of Sherloc's class 3 variants revealed a significant amount of uncertain variants with a likely pathogenic tendency (3 LP).

The C-terminal protein side harbours most (L)P variants

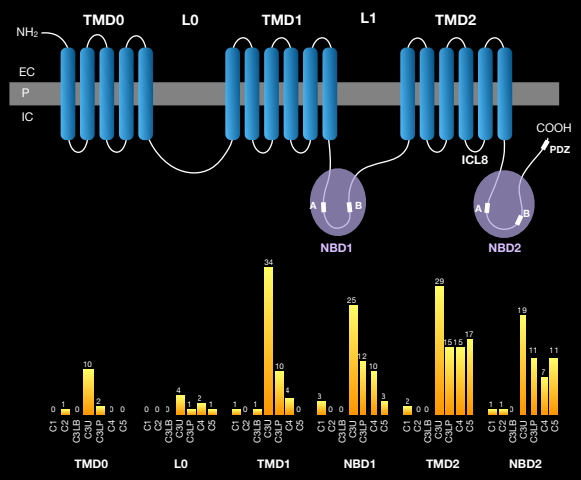

Fig 4. The transporter is characterised by two transmembrane domains (TMD), two linkers (L), two nucleotide binding domains (NBD) with Walker motifs (A and B) and a PDZ-like sequence. More variants reside in the C-terminal side of the protein, especially in TMD2 that contains the intracellular loop 8 (ICL8). This domain also seems to harbour more class 3 LP, class 4 and class 5 variants than any other domain.

References

Nykamp K et al. *Genetics in Medicine*. 2017,19(10):1105-1117. doi: 10.1038/gim.2017.37

Richards S et al. *Genetics in Medicine*. 2015,15(5):405-424. doi:10.1038/gim.2015.30

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